

Claims

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

same as
original
- #1
claim 1

1. (Currently Amended) A method of producing embryonic or stem-like cells, ~~wherein said cells comprise a nucleus derived from an adult differentiated cell and mitochondria from an oocyte of a species other than adult differentiated cell,~~ comprising the following steps:

(i) inserting a differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell or cell nucleus under conditions suitable for the formation of a nuclear transfer (NT) unit;

(ii) activating the resultant nuclear transfer (NT) unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) ~~disassociating said activated nuclear transfer unit; and~~

(iv) ~~isolating cells from said disassociated nuclear transfer unit~~ culturing cells from said nuclear transfer unit to obtain embryonic or stem-like cells.

2. (Currently Amended) The method of Claim 1, wherein the cell or cell nucleus inserted into the enucleated animal oocyte is a human cell or cell nucleus.

3. (Canceled)

4. (Currently Amended) The method of Claim 2, wherein said human cell or cell nucleus is an epithelial ~~cell~~, keratinocyte, lymphocyte, or fibroblast cell or cell nucleus.

5. (Currently Amended) The method of Claim 2, wherein the oocytes is ~~are~~ obtained from a mammal.

6. (Original) The method of Claim 5, wherein the animal oocyte is obtained from an ungulate.

7. (Original) The method of Claim 6, wherein said ungulate is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.

8. (Currently Amended) The method of Claim 1, wherein the ~~enucleated~~animal oocyte is matured prior to enucleation.

9. (Currently Amended) The method of Claim 1, wherein the ~~fused~~ nuclear transfer units ~~is are~~ activated *in vitro*.

10. (Currently Amended) The method of Claim 1, wherein the activated nuclear transfer units ~~are~~is cultured on a feeder layer ~~culture~~.

11. (Original) The method of Claim 10, wherein the feeder layer comprises fibroblasts.

12. (Canceled)

13. (Currently Amended) The method of Claim 12, wherein said feeder ~~cell~~ layer comprises fibroblasts.

14. (Original) The method of Claim 13, wherein said fibroblasts comprise mouse embryonic fibroblasts.

15. (Currently Amended) The method of Claim 1, wherein the resultant embryonic or stem-like cells are induced to differentiate.

16. (Currently Amended) The method of Claim 2, wherein the resultant embryonic or stem-like cells are induced to differentiate.

17. (Original) The method of Claim 1, wherein fusion is effected by electrofusion.

18. (Currently Amended) Embryonic or stem-like cells obtained according to the method of Claim 1.

19. (Currently Amended) Human embryonic or stem-like cells obtained according to the method of Claim 2.

20. (Canceled)

21. (Canceled)

22. (Canceled)

23. (Canceled)

24. (Original) Differentiated human cells obtained by the method of Claim 16.

25. (Original) The differentiated human cells of Claim 24, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.

26. (Withdrawn) A method of therapy which comprises administering to a patient in need of cell transplantation therapy isogenic differentiated human cells according to Claim 24.

~~27. (Withdrawn) The method of Claim 26, wherein said cell transplantation therapy is effected to treat a disease or condition selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, ALS, spinal cord defects or injuries, multiple sclerosis, muscular dystrophy, cystic fibrosis, liver disease, diabetes, heart disease, cartilage defects or injuries, burns, foot ulcers, vascular disease, urinary tract disease, AIDS and cancer.~~

~~28. (Withdrawn) The method of Claim 26, wherein the differentiated human cells are hematopoietic cells or neural cells.~~

~~29. (Withdrawn) The method of Claim 26, wherein the therapy is for the treatment of Parkinson's disease and the differentiated cells are neural cells.~~

~~30. (Withdrawn) The method of Claim 26, wherein the therapy is for the treatment of cancer and the differentiated cells are hematopoietic cells.~~

31. (Canceled)

32. (Currently Amended) The method of Claim 1, further comprising a step (v) whereby a gene is inserted, removed, or modified in said embryonic or stem-like cells.

33. (Previously Presented) The method of Claim 32, wherein said gene encodes a therapeutic enzyme, a growth factor, or a cytokine.

34. (Original) The method of Claim 32, wherein said embryonic or stem-like cells are human embryonic or stem-like cells.

35. (Previously Presented)) The method of claim 32, wherein said gene is removed, modified, or deleted by homologous recombination.

36. (Currently Amended) ~~The method of Claim 1,~~ A method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell or cell nucleus under conditions suitable for the formation of a nuclear transfer (NT) unit, and further wherein the donor differentiated human or mammalian cell or cell nucleus is genetically modified to impair the development of at least one of endoderm, ectoderm, and mesoderm;

(ii) activating the resultant nuclear transfer (NT) unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) culturing cells from said nuclear transfer unit to obtain embryonic or stem-like cells.

37. (Currently Amended) ~~The method of Claim 1,~~ A method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell or cell nucleus under conditions suitable for the formation of a nuclear transfer (NT) unit, and further wherein the ~~donor~~ differentiated human or mammalian cell or cell nucleus is genetically modified to increase differentiation efficiency;

(ii) activating the resultant nuclear transfer (NT) unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) culturing cells from said nuclear transfer unit to obtain embryonic or stem-like cells.

38. (Currently Amended) The method of Claim 36, wherein the ~~cultured~~ nuclear transfer unit is cultured in a media containing at least one ~~eapsase~~ caspase inhibitor.

39. (Canceled)

40. (Currently Amended) The method of Claim 36, wherein the ~~donor~~ differentiated human or mammalian cell or cell nucleus has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.

41. (Currently Amended) The method of Claim 37, wherein said differentiated human or mammalian cell or cell nucleus ~~donor cell~~ has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.

42. (Original) The method of Claim 41, wherein said gene or genes are operably linked to regulatable promoter.

43. (Currently Amended) ~~The method of Claim 1,~~ A method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell or cell nucleus under conditions suitable for the formation of a nuclear transfer (NT) unit, and further wherein the ~~donor~~ differentiated human or mammalian cell or cell nucleus is genetically modified to inhibit apoptosis;

(ii) activating the resultant nuclear transfer (NT) unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) culturing cells from said nuclear transfer unit to obtain embryonic or stem-like cells.

44. (Original) The method of Claim 43, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, GimL, Bid, EGL-1, Bcl-XL, Bcl-w, Mcl-1, A1, Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.

45. (Original) The method of Claim 44, wherein at least one of said genes is operably linked to an inducible promoter.

46. (Currently Amended) A mammalian somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is linked to a particular method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell or cell nucleus under conditions suitable for the formation of a nuclear transfer (NT) unit, and further wherein the ~~donor~~ differentiated human or mammalian cell or cell nucleus expresses a DNA construct encoding a cyclin that is operably linked to a gene that encodes a detectable marker;

(ii) activating the resultant nuclear transfer (NT) unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) culturing cells from said nuclear transfer unit to obtain embryonic or stem-like cells.

47. (Currently Amended) The differentiated human or mammalian cell or cell nucleus cell of Claim 46, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A, and H.

48. (Currently Amended) The differentiated human or mammalian cell or cell nucleus cell of Claim 46, wherein the detectable marker is a fluorescent polypeptide.

49. (Currently Amended) The differentiated human or mammalian cell or cell nucleus cell of Claim 48, wherein said cell or cell nucleus is selected from the group consisting of human, primate, rodent, ungulate, canine, and feline cells.

50. (Currently Amended) The differentiated human or mammalian cell or cell nucleus cell of Claim 48, wherein said cell is a human, bovine, or primate cell.

51. (Previously Presented) The embryonic or stem-like cells of Claim 32.

52. (Previously Presented) The method of Claim 1, wherein said differentiated cell and said enucleated oocyte are phylogenetically dissimilar.

53. (Canceled)

54. (Canceled)

55. (Canceled)

56. (Canceled)

57. (Canceled)

58. (Previously Presented) The method of claim 1, wherein said cells isolated from said disassociated nuclear transfer unit are isolated from cells originating from the inner-most portion of said nuclear transfer unit.

59. (Previously Presented) An embryonic stem-like cell isolated from the inner-most portion of a nuclear transfer unit according to the method of claim 58.

60. (Currently Amended) The method of claim 1, wherein the differentiated cell or cell nucleus ~~adult cell~~ inserted into the enucleated animal oocyte is a human cell, and the enucleated oocyte is a primary oocyte.

61. (Canceled)

WHAT IS CLAIMED IS:

1. A method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a desired differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell under conditions suitable for the formation of a nuclear transfer (NT) unit;

(ii) activating the resultant nuclear transfer unit;

(iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(iv) culturing cells obtained from said cultured NT units to obtain embryonic or stem-like cells.

2. The method of Claim 1, wherein the cell inserted into the enucleated animal oocyte is a human cell.

3. The method of Claim 2, wherein said human cell is an adult cell.

4. The method of Claim 2, wherein said human cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

5. The method of Claim 2, wherein the oocytes are obtained from a mammal.

6. The method of Claim 5, wherein the animal oocyte is obtained from an ungulate.

7. The method of Claim 6, wherein said ungulate is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.

8. The method of Claim 1, wherein the enucleated oocyte is matured prior to enucleation.

9. The method of Claim 1, wherein the fused nuclear transfer units are activated *in vitro*.

10. The method of Claim 1, wherein the activated nuclear transfer units are cultured on a feeder layer culture.

11. The method of Claim 10, wherein the feeder layer comprises fibroblasts.

12. The method of Claim 1, wherein in step (iv) cells from a NT unit having 16 cells or more are cultured on a feeder cell layer.

13. The method of Claim 12, wherein said feeder cell layer comprises fibroblasts.

14. The method of Claim 13, wherein said fibroblasts comprise mouse embryonic fibroblasts.

15. The method of Claim 1, wherein the resultant embryonic or stem-like cells are induced to differentiate.

16. The method of Claim 2, wherein the resultant embryonic or stem-like cells are induced to differentiate.

17. The method of Claim 1, wherein fusion is effected by electrofusion.

18. Embryonic or stem-like cells obtained according to the method of Claim 1.

19. Human embryonic or stem-like cells obtained according to the method of Claim 2.

20. Human embryonic or stem-like cells obtained according to the method of Claim 3.
21. Human embryonic or stem-like cells obtained according to the method of Claim 4.
22. Human embryonic or stem-like cells obtained according to the method of Claim 6.
23. Human embryonic or stem-like cells obtained according to the method of Claim 7.
24. Differentiated human cells obtained by the method of Claim 16.
25. The differentiated human cells of Claim 24, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.
26. A method of therapy which comprises administering to a patient in need of cell transplantation therapy isogenic differentiated human cells according to Claim 24.
27. The method of Claim 26, wherein said cell transplantation therapy is effected to treat a disease or condition selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, ALS, spinal cord defects or injuries, multiple sclerosis, muscular dystrophy, cystic fibrosis, liver disease, diabetes, heart disease, cartilage defects or injuries, burns, foot ulcers, vascular disease, urinary tract disease, AIDS and cancer.
28. The method of Claim 26, wherein the differentiated human cells are hematopoietic cells or neural cells.

29. The method of Claim 26, wherein the therapy is for treatment of Parkinson's disease and the differentiated cells are neural cells.

30. The method of Claim 26, wherein the therapy is for the treatment of cancer and the differentiated cells are hematopoietic cells.

31. The differentiated human cells of Claim 24, which contain and express an inserted gene.

32. The method of Claim 1, wherein a desired gene is inserted, removed or modified in said embryonic or stem-like cells.

33. The method of Claim 32, wherein the desired gene encodes a therapeutic enzyme, a growth factor or a cytokine.

34. The method of Claim 32, wherein said embryonic or stem-like cells are human embryonic or stem-like cells.

35. The method of Claim 32, wherein the desired gene is removed, modified or deleted by homologous recombination.

36. The method of Claim 1, wherein the donor cell is genetically modified to impair the development of at least one of endoderm, ectoderm and mesoderm.

37. The method of Claim 1, wherein the donor cell is genetically modified to increase differentiation efficiency.

38. The method of Claim 36, wherein the cultured nuclear transfer unit is cultured in a media containing at least one caspase inhibitor.

39. The method of Claim 1, wherein the donor cell expresses a detectable label that is indicative of the expression of a particular cyclin.

40. The method of Claim 36, wherein the donor cell has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.

41. The method of Claim 37, wherein said donor cell has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.

42. The method of Claim 41, wherein said gene or genes are operably linked to a regulatable promoter.

43. The method of Claim 1, wherein the donor cell has been genetically modified to inhibit apoptosis.

44. The method of Claim 43, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, GimL, Bid, EGL-1, Bcl-XL, Bcl-w, Mcl-1, A1, Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.

45. The method of Claim 44, wherein at least one of said genes is operably linked to an inducible promoter.

46. A mammalian somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is linked to a particular cyclin.

47. The cell of Claim 46, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A and H.

48. The cell of Claim 46, wherein the detectable marker is a fluorescent polypeptide.

49. The cell of Claim 48, wherein said mammalian cell is selected from the group consisting of human, primate, rodent, ungulate, canine, and feline cells.

50. The cell of Claim 48, wherein said cell is a human, bovine or primate cell.

51. A method of producing human embryonic stem-like cells, comprising:

- (i) inserting a human cell or cell nucleus into a recipient oocyte that is derived from a non-human mammal, under conditions suitable for the formation of a nuclear transfer (NT) unit;
- (ii) activating the resultant NT unit;
- (iii) culturing the activated NT unit until greater than the 2-cell developmental stage; and
- (iv) culturing cells obtained from the cultured NT unit to obtain human embryonic stem-like cells.

52. The method of Claim 51, wherein the recipient oocyte is enucleated prior to inserting the human cell or cell nucleus.

53. The method of Claim 51, wherein the oocyte comprises a protein or other substance that improves reprogramming efficiency or limits the differentiation potential of the NT unit.

54. The method of Claim 52, wherein the recipient oocyte is a rabbit oocyte.

55. The method of Claim 54, wherein the recipient oocyte is spiked with rabbit ooplasm.

56. The method of Claim 55, wherein the oocyte comprises a protein or other substance that improves reprogramming efficiency or limits the differentiation potential of the nuclear transfer unit.

57. The method of Claim 51, wherein the activated NT unit is cultured until the blastocyst stage.

58. A pluripotent human embryonic stem-like cell made by the method of claim 51.

59. The pluripotent human embryonic stem-like cell of Claim 58, which cell contains mitochondrial DNA of the recipient oocyte.

60. A pluripotent human embryonic stem-like cell made by the method of Claim 54.

61. The pluripotent human embryonic stem-like cell of Claim 60, which cell contains rabbit mitochondrial DNA.

62. A method for producing a nuclear transfer human embryo by cross-species nuclear transfer comprising:

- (i) transferring a human cell or human nucleus into a rabbit oocyte which is enucleated before, simultaneous or after said human cell or nuclear transfer; and
- (ii) the resultant fusion is permitted to develop into a nuclear transfer embryo.

63. The method of Claim 62 which comprises transferring the pronucleus that results after step (ii) into another oocyte which is enucleated before, simultaneous or after transfer.

64. The method of Claim 62 which further comprises transferring additional ooplasm from another rabbit oocyte into the resultant fusion.

65. The method of Claim 62 wherein the resultant fusion is activated simultaneous to fusion to induce embryonic development.

66. The method of Claim 62 wherein the rabbit oocyte is activated prior to transferral of said human cell or nucleus therein.
67. The method of claim 62 which includes an activation step effected after said human cell or human nuclear transfer.
68. The method of Claim 65, 66 or 67 wherein activation includes the use of DMAP and cycloheximide.
69. The method of Claim 62 wherein the nuclear transfer embryo produced by step (ii) is cultured on a feeder layer.
70. The method of Claim 69 wherein said culture gives rises to human pluripotent cells.
71. The method of Claim 70 wherein said pluripotent cells are permitted to differentiate into different cell types.
72. The method of Claim 69 wherein the embryo is cultured on a fibroblast feeder layer.
73. A method for enhancing the development of a cross-species nuclear transfer unit produced by transferral of a human cell or nucleus into a non-human oocyte, comprising introducing into said oocyte or said human cell prior to transferral ooplasm from a rabbit oocyte.
74. The method of Claim 73 wherein said non-human oocyte is an ungulate oocyte.
75. The method of Claim 74 wherein said ungulate oocyte is a bovine oocyte.

76. A cross-species blastula or morula that results from the process of Claim 62 or Claim 73.

77. Differentiated cells derived from the morula or blastocyst of Claim 76.

original
for comparison

WHAT IS CLAIMED IS:

Sub A.
X A method of producing embryonic or stem-like cells comprising the following steps:

- (i) inserting a desired differentiated human or mammalian cell or cell nucleus into an enucleated animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell under conditions suitable for the formation of a nuclear transfer (NT) unit;
- (ii) activating the resultant nuclear transfer unit;
- (iii) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and
- (iv) culturing cells obtained from said cultured NT units to obtain embryonic or stem-like cells.

2. The method of Claim 1, wherein the cell inserted into the enucleated animal oocyte is a human cell.

3. The method of Claim 2, wherein said human cell is an adult cell.

4. The method of Claim 2, wherein said human cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

5. The method of Claim 2, wherein the oocytes are obtained from a mammal.

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This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A method of producing ungulate embryonic stem-like cells, wherein said cells comprise a nucleus derived from an adult differentiated cell of a first ungulate species and mitochondria from an oocyte of a second ungulate species other than the species of said adult differentiated cell, comprising the following steps:

- (i) inserting a donor differentiated cell or cell nucleus of said first ungulate species into a recipient animal oocyte of said second ungulate species under conditions suitable for the formation of a nuclear transfer (NT) unit, wherein the endogenous oocyte nucleus is removed or inactivated before, concurrent, or after introduction of donor cell or nucleus;
- (ii) activating the resultant nuclear transfer unit;
- (iii) additionally inserting into said oocyte cytoplasm derived from a second oocyte or a blastomere of the same species as the donor cell or nucleus;
- (iv) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage;
- (v) dissociating said activated nuclear transfer unit; and
- (vi) isolating cells having the nuclear material of said first ungulate species and the mitochondria of said second different ungulate species from said disassociated nuclear transfer unit to obtain embryonic stem-like cells.

2. (Previously amended) The method of claim 1, which further includes introducing the mitochondrial DNA of the same species as the donor cell or nucleus into the recipient oocyte.

3. (Original) The method of Claim 1, wherein said cytoplasm is introduced before, concurrent, or after introduction of donor cell or nucleus.

4. (Currently amended) The method of claim 4 3, wherein said introduction occurs within about six hours of introduction of the donor cell or nucleus.

5. (Previously amended) The method of Claim 4, wherein said second oocyte is an immature oocyte.

6. (Previously amended) The method of Claim 5, wherein said second oocyte is an immature bovine oocyte.

7. (Previously amended) The method of Claim 5, wherein said immature oocyte is matured *in vitro* prior to isolation of cytoplasm therefrom.

8. (Previously amended) The method of Claim 5, wherein said immature oocyte is activated *in vitro* prior to isolation of cytoplasm therefrom.

9. (Original) The method of Claim 8, wherein said *in vitro* activation comprises contacting said oocyte with a compound that increases calcium levels.

10. (Previously amended) The method of Claim 2, wherein all or part of the cytoplasm of the recipient oocyte is removed prior to introduction of cytoplasm from said at least one second oocyte or blastomere of the same species as the donor cell or nucleus.

11. (Previously amended) The method of Claim 1, wherein the cell or cell nucleus inserted into the enucleated oocyte is a bovine cell.

12. (Previously amended) The method of Claim 11, wherein said bovine cell is an adult cell.

13. (Previously amended) The method of Claim 11, wherein said bovine cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

14. (Previously amended) The method of Claim 11, wherein the recipient oocyte is obtained from a bovine mammal.

15. (Previously amended) The method of Claim 14, wherein the animal oocyte is obtained from *Bos taurus*.

16. (Previously amended) The method of Claim 1, wherein said first and second ungulate species are both of an ungulate that is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.

17. (Original) The method of Claim 1, wherein the enucleated oocyte is matured prior to enucleation.

18. (Previously amended) The method of Claim 1, wherein the fused nuclear transfer unit is activated *in vitro*.

19. (Previously amended) The method of Claim 1, wherein the activated nuclear transfer unit is cultured on a feeder layer culture.

20. (Original) The method of Claim 19, wherein the feeder layer comprises fibroblasts.

21. (Original) The method of claim 1, wherein in step (v) cells from a NT unit having 16 cells or more are cultured on a feeder cell layer.

22. (Original) The method of Claim 21, wherein said feeder cell layer comprises fibroblasts.

23. (Original) The method of claim 22, wherein said fibroblasts comprise mouse embryonic fibroblasts.

24. (Previously amended) The Method of Claim 1, wherein the resultant embryonic stem-like cells are induced to differentiate.

25. (Previously amended) The method of Claim 11, wherein the resultant bovine embryonic stem-like cells are induced to differentiate.

26. (Original) The method of Claim 1, wherein fusion is effected by electrofusion.

27. (Currently amended) Ungulate embryonic stem-like cells obtained according to the method of Claim 1, which cells have mitochondria of said second ungulate species.

28. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 11, which cells have mitochondria of said second ungulate species.

29. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 12, which cells have mitochondria of said second ungulate species.

30. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 13, which cells have mitochondria of said second ungulate species.

31. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 14, which cells have mitochondria of said second ungulate species.

32. (Previously amended) Bovine embryonic stem-like cells obtained according to the method of Claim 15, which cells have mitochondria of said second ungulate species.

33. (Previously amended) Differentiated bovine cells obtained by the method of Claim 25, which cells have mitochondria of said second ungulate species.

34. (Previously amended) The differentiated bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells, which cells have mitochondria of said second ungulate species.

35. (Canceled)

36. (Previously amended) The method of Claim 1, further comprising a step whereby a desired gene is inserted, removed or modified in said embryonic stem-like cells.

37. (Original) The method of Claim 36, wherein the desired gene encodes a therapeutic enzyme, a growth factor or a cytokine.

38. (Previously amended) The method of Claim 37, wherein said embryonic stem-like cells are bovine embryonic stem-like cells.

39. (Original) The method of Claim 36, wherein the desired gene is removed, modified or deleted by homologous recombination.

40. (Original) The method of Claim 1, wherein the donor cell is genetically modified to impair the development of at least one of endoderm, ectoderm and mesoderm.

41. (Original) The method of Claim 1, wherein the donor cell is genetically modified to increase differentiation efficiency.

42. (Original) The method of Claim 40, wherein wherein the cultured nuclear transfer unit is cultured in a media containing at least one caspase inhibitor.

43. (Original) The method of Claim 1, wherein the donor cell expresses a detectable label that is indicative of the expression of a particular cyclin.

44. (Original) The method of Claim 40, wherein the donor cell has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.

45. (Original) The method of Claim 41, wherein said donor cell has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.

46. (Original) The method of Claim 45, wherein said gene or genes are operably linked to a regulatable promoter.

47. (Original) The method of Claim 1, wherein the donor cell has been genetically modified to inhibit apoptosis.

48. (Original) The method of Claim 47, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, GimL, Bid, EGL-1, Bcl-CL, Bcl-w, Mcl-1, A1, Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.

49. (Original) The method of Claim 48, wherein at least one of said genes is operably linked to an inducible promoter.

50. (Previously amended) An ungulate somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is operably linked to a promoter that regulates the expression of a particular cyclin.

51. (Original) The cell of Claim 50, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A and H.

52. (Original) The cell of Claim 50, wherein the detectable marker is a fluorescent polypeptide.

53. (Original) The cell of Claim 52, wherein said mammalian cell is selected from the group consisting of human, primate, rodent, ungulate, canine, and feline cells.

54. (Original) The cell of Claim 50, wherein said cell is a human, bovine or primate cell.

55. (Previously amended) The method of claim 1, wherein said cells isolated from said disassociated nuclear transfer unit are isolated from cells originating from the inner-most portion of said nuclear transfer unit.

56. (Previously amended) An ungulate embryonic stem-like cell isolated from the inner-most portion of a nuclear transfer unit according to the method of claim 55, which cell has mitochondria of said second ungulate species.

57. (Previously amended) Differentiated bovine cells obtained by the method of Claim 33, wherein said differentiated cells contain and express and inserted gene.

58. (Previously amended) The differentiated bovine cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.

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- (ii) activating the resultant nuclear transfer unit;
 - (iii) culturing said activated nuclear transfer units until greater than the 2-cell developmental stage; and
 - (iv) culturing cells obtained from said cultured NT units to obtain embryonic stem-like cells.
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15. (Amended) The method of Claim 1, wherein the resultant embryonic stem-like cells are induced to differentiate.

16. (Amended) The method of Claim 2, wherein the resultant embryonic stem-like cells are induced to differentiate.

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18. (Amended) Embryonic stem-like cells according to the method of Claim 1.

19. (Amended) Human embryonic stem-like cells according to the method of Claim 2.

21. (Amended) Human embryonic stem-like cells according to the method of Claim 4.

A⁴

22. (Amended) Human embryonic stem-like cells according to the method of Claim 6.

23. (Amended) Human embryonic stem-like cells according to the method of claim 7.

A⁵

32. (Amended) The method of Claim 1, further comprising a step (v) whereby a gene is inserted, removed or modified in said embryonic stem-like cells.

33. (Amended) The method of Claim 32, wherein said gene encodes a therapeutic enzyme, a growth factor or a cytokine.
